

# SEQUENCE LISTING

<110> Leng, Jay

<120> PROTEASE SPECIFIC CLEAVABLE LUCIFERASES AND METHODS OF  
USE THEREOF

<130> 105175-159907

<140> Not Yet Known

<141> 2000-07-19

<160> 29

<170> PatentIn Ver. 2.1

<210> 1

<211> 936

<212> DNA

<213> Renilla reniformis

<220>

<221> CDS

<222> (1) .. (936)

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atg act tcg aaa gtt tat gat cca gaa caa agg aaa cgg atg ata act	48
Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr	
1 5 10 15	
ggt ccg cag tgg tgg gcc aga tgt aaa caa atg aat gtt ctt gat tca	96
Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser	
20 25 30	
ttt att aat tat tat gat tca gaa aaa cat gca gaa aat gct gtt att	144
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile	
35 40 45	
ttt tta cat ggt aac gcg gcc tct tct tat tta tgg cga cat gtt gtg	192
Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val	
50 55 60	
cca cat att gag cca gta gcg cgg tgt att ata cca gat ctt att ggt	240
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly	
65 70 75 80	
atg ggc aaa tca ggc aaa tct ggt aat ggt tct tat agg tta ctt gat	288
Met, Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp	

85

90

95

cat tac aaa tat ctt act gca tgg ttt gaa ctt ctt aat tta cca aag 336  
 His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
 100 105 110

aag atc att ttt gtc ggc cat gat tgg ggt gct tgt ttg gca ttt cat 384  
 Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His  
 115 120 125

tat agc tat gag cat caa gat aag atc aaa gca ata gtt cac gct gaa 432  
 Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu  
 130 135 140

agt gta gta gat gtg att gaa tca tgg gat gaa tgg cct gat att gaa 480  
 Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
 145 150 155 160

gaa gat att gcg ttg atc aaa tct gaa gaa gga gaa aaa atg gtt ttg 528  
 Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
 165 170 175

gag aat aac ttc ttc gtg gaa acc atg ttg cca tca aaa atc atg aga 576  
 Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg  
 180 185 190

aag tta gaa cca gaa gaa ttt gca gca tat ctt gaa cca ttc aaa gag 624  
 Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
 195 200 205

aaa ggt gaa gtt cgt cgt cca aca tta tca tgg cct cgt gaa atc ccg 672  
 Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
 210 215 220

tta gta aaa ggt ggt aaa cct gac gtt gta caa att gtt agg aat tat 720  
 Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
 225 230 235 240

aat gct tat cta cgt gca agt gat gat tta cca aaa atg ttt att gaa 768  
 Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu  
 245 250 255

tcg gat cca gga ttc ttt tcc aat gct att gtt gaa ggc gcc aag aag 816  
 Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
 260 265 270

ttt cct aat act gaa ttt gtc aaa gta aaa ggt ctt cat ttt tcg caa 864  
 Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln

275	280	285	
gaa gat gca cct gat gaa atg gga aaa tat atc aaa tcg ttc gtt gag 912			
Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu			
290	295	300	

cga gtt ctc aaa aat gaa caa taa 936
Arg Val Leu Lys Asn Glu Gln
305 310

<210> 2  
 <211> 311  
 <212> PRT  
 <213> Renilla reniformis

<400> 2

Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr	
1 5 10 15	
Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser	
20 25 30	
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile	
35 40 45	
Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val	
50 55 60	
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly	
65 70 75 80	
Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp	
85 90 95	
His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys	
100 105 110	
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His	
115 120 125	
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu	
130 135 140	
Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu	
145 150 155 160	
Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu	
165 170 175	
Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg	
180 185 190	
Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu	
195 200 205	
Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro	
210 215 220	
Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr	
225 230 235 240	
Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu	



100	105	110	
aag atc att ttt gtc ggc cat gat tgg ggt gct tgt ttg gca ttt cat			384
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His			
115	120	125	
tat agc tat gag cat caa gat aag atc aaa gca ata gtt cac gct gaa			432
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu			
130	135	140	
agt gta gta gat gtg att gaa tca tgg gat gaa tgg cct gat att gaa			480
Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu			
145	150	155	160
gaa gat att gcg ttg atc aaa tct gaa gaa gga gaa aaa atg gtt ttg			528
Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu			
165	170	175	
gag aat aac ttc ttc gtg gaa acc atg ttg cca tca aaa atc atg aga			576
Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg			
180	185	190	
aag tta gaa cca gac gaa gtt gac gca tat ctt gaa cca ttc aaa gag			624
Lys Leu Glu Pro Asp Glu Val Asp Ala Tyr Leu Glu Pro Phe Lys Glu			
195	200	205	
aaa ggt gaa gtt cgt cgt cca aca tta tca tgg cct cgt gaa atc ccg			672
Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro			
210	215	220	
tta gta aaa ggt ggt aaa cct gac gtt gta caa att gtt agg aat tat			720
Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr			
225	230	235	240
aat gct tat cta cgt gca agt gat gat tta cca aaa atg ttt att gaa			768
Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu			
245	250	255	
tcg gat cca gga ttc ttt tcc aat gct att gtt gaa ggc gcc aag aag			816
Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys			
260	265	270	
ttt cct aat act gaa ttt gtc aaa gta aaa ggt ctt cat ttt tcg caa			864
Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln			
275	280	285	
gaa gat gca cct gat gaa atg gga aaa tat atc aaa tcg ttc gtt gag			912
Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu			

290

295

300

cga gtt ctc aaa aat gaa caa taa  
 Arg Val Leu Lys Asn Glu Gln  
 305 310

936

&lt;210&gt; 4

&lt;211&gt; 311

&lt;212&gt; PRT

&lt;213&gt; Renilla reniformis (mutated sequence)

&lt;400&gt; 4

Met	Thr	Ser	Lys	Val	Tyr	Asp	Pro	Glu	Gln	Arg	Lys	Arg	Met	Ile	Thr
1				5					10					15	
Gly	Pro	Gln	Trp	Trp	Ala	Arg	Cys	Lys	Gln	Met	Asn	Val	Leu	Asp	Ser
			20					25					30		
Phe	Ile	Asn	Tyr	Tyr	Asp	Ser	Glu	Lys	His	Ala	Glu	Asn	Ala	Val	Ile
		35					40					45			
Phe	Leu	His	Gly	Asn	Ala	Ala	Ser	Ser	Tyr	Leu	Trp	Arg	His	Val	Val
	50					55					60				
Pro	His	Ile	Glu	Pro	Val	Ala	Arg	Cys	Ile	Ile	Pro	Asp	Leu	Ile	Gly
65					70					75				80	
Met	Gly	Lys	Ser	Gly	Lys	Ser	Gly	Asn	Gly	Ser	Tyr	Arg	Leu	Leu	Asp
				85					90					95	
His	Tyr	Lys	Tyr	Leu	Thr	Ala	Trp	Phe	Glu	Leu	Leu	Asn	Leu	Pro	Lys
			100					105					110		
Lys	Ile	Ile	Phe	Val	Gly	His	Asp	Trp	Gly	Ala	Cys	Leu	Ala	Phe	His
		115					120					125			
Tyr	Ser	Tyr	Glu	His	Gln	Asp	Lys	Ile	Lys	Ala	Ile	Val	His	Ala	Glu
	130					135					140				
Ser	Val	Val	Asp	Val	Ile	Glu	Ser	Trp	Asp	Glu	Trp	Pro	Asp	Ile	Glu
145					150					155				160	
Glu	Asp	Ile	Ala	Leu	Ile	Lys	Ser	Glu	Glu	Gly	Glu	Lys	Met	Val	Leu
				165					170					175	
Glu	Asn	Asn	Phe	Phe	Val	Glu	Thr	Met	Leu	Pro	Ser	Lys	Ile	Met	Arg
			180					185					190		
Lys	Leu	Glu	Pro	Asp	Glu	Val	Asp	Ala	Tyr	Leu	Glu	Pro	Phe	Lys	Glu
		195					200						205		
Lys	Gly	Glu	Val	Arg	Arg	Pro	Thr	Leu	Ser	Trp	Pro	Arg	Glu	Ile	Pro
	210					215					220				
Leu	Val	Lys	Gly	Gly	Lys	Pro	Asp	Val	Val	Gln	Ile	Val	Arg	Asn	Tyr
225					230					235				240	
Asn	Ala	Tyr	Leu	Arg	Ala	Ser	Asp	Asp	Leu	Pro	Lys	Met	Phe	Ile	Glu
				245					250					255	
Ser	Asp	Pro	Gly	Phe	Phe	Ser	Asn	Ala	Ile	Val	Glu	Gly	Ala	Lys	Lys
		260						265					270		
Phe	Pro	Asn	Thr	Glu	Phe	Val	Lys	Val	Lys	Gly	Leu	His	Phe	Ser	Gln

	275		280		285
Glu	Asp	Ala	Pro	Asp	Glu
		Met	Gly	Lys	Tyr
			Ile	Lys	Ser
				Phe	Val
				Glu	
290		295		300	
Arg	Val	Leu	Lys	Asn	Gln
305		310			

<210> 5  
 <211> 8  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Protease  
 recognition sequences

<400> 5  
 Ser Gln Asn Tyr Pro Ile Val Gln  
 1 5

<210> 6  
 <211> 10  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Protease  
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<400> 6  
 Lys Ala Arg Val Leu Ala Glu Ala Met Ser  
 1 5 10

<210> 7  
 <211> 10  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Protease  
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<400> 7  
 Pro Ser Pro Arg Glu Gly Lys Arg Ser Tyr  
 1 5 10

<210> 8  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 8  
Tyr Val Ala Asp Gly  
1 5

<210> 9  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 9  
Met Phe Gly Gly Ala Lys Lys Arg  
1 5

<210> 10  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 10  
Gly Val Val Asn Ala Ser Ser Arg Leu Ala  
1 5 10

<210> 11  
<211> 9  
<212> PRT  
<213> Artificial Sequence



<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 11

Leu Ile Ala Tyr Leu Lys Lys Ala Thr

1

5

<210> 12

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 12

Val Lys Met Asp Ala Glu Phe

1

5

<210> 13

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 13

Phe Leu Ala Glu Gly Gly Gly Val Arg Gly Pro Arg Val Val Glu Arg

1

5

10

15

His

<210> 14

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease

recognition sequences

<400> 14

Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His

1

5

10

<210> 15

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 15

Lys Pro Ala Leu Phe Phe Arg Leu

1

5

<210> 16

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 16

Ile Glu Pro Asp

1

<210> 17

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 17

Asp Glu Thr Asp

1

<210> 18  
<211> 4  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: Protease  
recognition sequences  
  
<400> 18  
Trp Glu His Asp  
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<210> 19  
<211> 4  
<212> PRT  
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recognition sequences  
  
<400> 19  
Tyr Val Ala Asp  
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<210> 20  
<211> 4  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
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recognition sequences  
  
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Asp Glu His Asp  
1

<210> 21  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

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Asp Glu Val Asp

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<210> 22

<211> 4

<212> PRT

<213> Artificial Sequence

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<223> X at residue 1 is W or L

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

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Xaa Glu His Asp

1

<210> 23

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> X at residue 3 is I or H

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 23

Val Glu Xaa Asp

1

<210> 24

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 24

Leu Glu Thr Asp

1

<210> 25

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 25

Leu Glu His Asp

1

<210> 26

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> X at residues 1 to 3 can be any amino acid

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequences

<400> 26

Xaa Xaa Xaa Asp

1

<210> 27

<211> 8

<212> PRT

<213> Artificial Sequence

<400> 27

Arg Pro Leu Gly Ile Ile Gly Gly

1

5

<210> 28

<211> 3

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequence

<400> 28

Glu Gly Arg

1

<210> 29

<211> 3

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Protease  
recognition sequence

<400> 29

Val Leu Lys

1